

The University of Chicago

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TRICHINELLA SPIRALIS

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDI-
DACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY
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Flury,¹ in 1913, suggested the presence of glucoprotein in the larvae of *Trichinella spiralis*. However, no studies of the antigenicity of this component were made. The purpose of the present investigation was to isolate and identify those chemical fractions of the larvae which are immunologically active.

Extracts of the larvae of *Trichinella spiralis* have been used in immunological reactions by several investigators since Ströbel² in 1911 first detected complement-fixing antibodies in the serum of trichinella-infected animals. His antigen was prepared by mincing trichinous muscle, digesting it with pepsin and hydrochloric acid, centrifugalizing, and then washing the sediment. This sediment, containing the free parasites and undigested muscle, was dried, extracted with 0.1 N NaOH or antiformin, and the extract neutralized.

Romanovitch,³ working with a saline extract of digested muscle-parasite mass and Ducas,⁴ using aqueous extracts of infected meat, both reported inconclusive results with the complement-fixation and precipitin tests. Positive precipitin and skin tests were never demonstrated consistently until Bachman^{5,6,7}

in 1928 reported his results. He used the method of Ströbel, but obtained living trichinae that had been freed from host protein. The isolated worms were dried, powdered, and the lipoids removed by ether extraction. The ether-insoluble portion was centrifugalized, hydrolyzed by 0.1% HCl in saline, and the supernatant of the hydrolysate adjusted to pH 7.2-7.4 before being used for precipitin reactions. A Coca's extract of the larvae was employed in skin testing. Bachman's success was probably due to the recovery of soluble antigen, or antigens, of larvae completely freed from host tissue, whereas earlier workers employed extracts of incompletely digested infected muscle. Augustine and Theiler⁸ evaluated the application of these tests to infected humans and animals, and concluded that the skin test and precipitation reaction are of specific value in the diagnosis of trichinosis. Many reports have appeared confirming these results.⁹⁻¹⁸

6. Bachman, G. W.: J. Prev. Med. 2: 169, 1928.

7. Bachman, G. W.: J. Prev. Med. 2: 513, 1928.

8. Augustine, D. L., and Theiler, H.: Parasitology 24: 60, 1932.

9. Hunter, G. W.: Am. J. Hyg. 13: 311, 1931.

10. Swineford, O., and Waddell, W. W.: Virginia M. Monthly 59: 28, 1932.

11. Maternowska, I.: Zentralbl. f. Bakt. 129: 284, 1933.

12. Friedlander, R. D.: Am. J. M. Sc. 188: 121, 1934.

13. Sobel, I. P.: Am. J. Dis. Child. 51: 367, 1936.

14. Goldschlager, A. I.: Ann. Int. Med. 8: 939, 1935.

15. Drake, E. A., Hawkes, R. S., and Warren, M.: J.A.M.A. 105: 1340, 1935.

16. Spink, W. W., and Augustine, D. L.: J.A.M.A. 104: 1801, 1935.

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1. Flury, F.: Arch. f. Exper. Path. u. Pharmacol. 73: 164, 1913.

2. Ströbel, H.: München. med. Wchnschr. 58: 672, 1911.

3. Romanovitch, M.: Ann. Inst. Pasteur. 26: 351, 1912.

4. Ducas, R.: L'immunité dans la trichinose. Thèse Jouve et Cie, Paris, p. 47, 1921.

5. Bachman, G. W.: J. Prev. Med. 2: 35, 1928.

In 1933, McCoy *et al.*¹⁹ modified Bachman's procedure of extraction by using a phosphate-buffered saline solution. They found that their intradermal test possessed a fairly high degree of specificity.

In 1938, Bozicevich²⁰ prepared a saline extract of desiccated larvae, for which he claimed a longer-lasting potency. He stated that his extract gave fewer non-specific reactions because only sodium chloride was used in the extraction. This antigen was used for precipitin and intradermal tests in an outbreak of trichinosis²¹ and was found satisfactory.

Oliver-González,²² in 1941, demonstrated the existence of a specific antibody against the adult form of the parasite. No attempt is made in the present work to obtain antigens from this stage of *Trichinella*.

MATERIALS AND METHODS

Larvae were obtained from two 80 kg. hogs experimentally infected one month prior to killing. The carcasses were skinned, eviscerated, and the bones removed. One kg. lots of the heavily infected muscle were then minced and digested in 15 liters of 0.5% HCl and 0.7% pepsin (Pepsin, U.S.P.). The mixture was agitated constantly with a mechanical stirrer for 2 hours at 38 C. The larvae settled out and were passed first through 20-per-inch and then through 60-per-inch wire meshes to

remove bone particles, cartilage, and undigested muscle strands. All undigested muscle was redigested subsequently. The larvae were washed repeatedly in sterile saline until the washings gave negative biuret tests, indicating that the worms were free of host protein. The vessel containing the packed worms was then immersed in a freezing mixture of solid carbon dioxide and methyl "Cellosolve" (−72 C.) and the larvae were frozen within 1–3 minutes. Larvae recovered from each digestion were treated similarly and then stored in a chest refrigerated with solid carbon dioxide until all the digestions were completed. When all the infected meat was digested the recovered frozen larvae were placed in a lyophilizing condenser and lyophilized. This method of drying in the frozen state prevents autolytic changes, and the dehydrated material in lyophilized form has a porosity which makes it particularly well adapted to rapid and thorough extraction with many lipid solvents. Furthermore, proteins in a lyophilized state are much less subject to denaturation, and therefore their antigenic structure is less liable to be altered.

Isolation of the Polysaccharide Fraction

A preliminary report concerning this polysaccharide fraction has already been published.²³ The method used was similar to that employed in the extraction of polysaccharides from *Ascaris*²⁴ and other parasitic helminthes.^{25,26} Three gms. of lyophilized material were pulverized by mortar and pestle, sus-

17. Theiler, A., Augustine, D. L., and Spink, W. W.: *Parasitology* **27**: 345, 1935.

18. Spink, W. W.: *New England J. Med.* **216**: 5, 1937.

19. McCoy, O. R., Miller, J. J., and Friedlander, R. D.: *J. Immunol.* **24**: 1, 1933.

20. Bozicevich, J.: *Pub. Health Rep.* **53**: 2130, 1938.

21. Ferenbaugh, T. L., Segal, L., and Schulze, H. A.: *J.A.M.A.* **110**: 1434, 1938.

22. Oliver-González, J.: *J. Infect. Dis.* **69**: 254, 1941.

23. Melcher, L. R., and Campbell, D. H.: *Science* **96**: 431, 1942.

24. Campbell, D. H.: *J. Infect. Dis.* **59**: 266, 1936.

25. Campbell, D. H.: *J. Infect. Dis.* **65**: 12, 1939.

26. Campbell, D. H.: *J. Parasitol.* **23**: 348, 1937.

pended in 20 volumes of 0.1 N borate buffer, pH 8.0, placed in a boiling water bath, and stirred vigorously with a mechanical stirrer. After 30 minutes the mixture was removed, cooled, and centrifugalized. The residue was washed once with the buffer and the washing was added to the original extract. The polysaccharide and other soluble worm materials were then precipitated by the addition of 5 volumes of chilled 95% ethanol. In order to facilitate precipitation by ethanol, enough NaCl was added to give a concentration of approximately 0.5%. After 4 hours at 4 C., a white gummy precipitate that had formed was removed by centrifugalization and carefully resuspended in approximately 20 volumes of pH 4.6 acetate buffer. The material which failed to redissolve was removed and discarded. The solution, which contained mostly polysaccharide, was adjusted to pH 8.0 by the addition of 1.0 N sodium carbonate solution. The polysaccharide was again precipitated by the addition of NaCl solution and 2 volumes of 95% ethanol. Reprecipitation at pH 8.0 and redissolving at pH 4.6 was repeated until a preparation was obtained which failed to give the usual tests for protein or amino acids, namely, biuret, ninhydrin, xanthoproteic, Millon's and sulfosalicylic acid tests. Five to 8 treatments were required to obtain a completely soluble product at pH 4.6. The polysaccharide was finally reprecipitated with ethanol and dried several times with absolute ethanol and ether. Approximately 300 mgs. of polysaccharide were obtained from 3.0 gms. of lyophilized whole worm material.

The resulting product was a fine white powder which dissolved readily in water, giving an opalescent solution in low dilutions. This solution failed to diffuse through cellophane membranes. In di-

lutions of 1:10,000 it gave a positive Molisch reaction. A test for nitrogen by Nessler's method on a 10 mg. sample was negative. No reducing property was observed before acid hydrolysis, but appeared rapidly on treatment with 1.0 N HCl solution. Its immunological properties were not affected by autoclaving for 15 minutes at 15 lbs. pressure at pH 7.0.

Isolation of Protein Fractions

Two gms. of the lyophilized, pulverized larval material were treated with petroleum ether in a Soxhlet apparatus for a week, in order to extract lipoids. The petroleum ether solvent was separated from the lipoids by vacuum distillation. The insoluble residue was an oily, brown substance, weighing 70 mgs., or 3.5 % of the dehydrated worm material. Until ready for use, the ether-soluble lipoids were kept at 4 C. in a vacuum desiccator containing anhydrous calcium sulfate as the desiccant.

The removal of lipoids from the larval tissue facilitated the extraction of proteins. The petroleum ether-insoluble residue was next triturated in 0.1 N borate buffer, pH 8.3, small amounts (50 ml. in all) being added at a time. This suspension was then stirred slowly at 4 C. for 12 hours. To prevent denaturation of the proteins due to too rapid mechanical agitation, a motor making 60 revolutions per minute was used. This triturated material was then centrifugalized for an hour at 18,000 revolutions per minute to remove any insoluble substances. A clear, brownish-yellow supernatant and a residue were obtained. The supernatant was decanted from the residue, which was then washed twice with the borate buffer. The washings were added to the supernatant, which was labelled Supernatant I. The residue was lyophilized and stored in vacuo until ready for use (see fig. 1). Half of Supernatant I was ad-

justed to pH 4.8 with 0.2 N HCl, and a white precipitate was obtained. This precipitate was separated from its supernatant (henceforth known as Supernatant II) by centrifugation, the Supernatant II retaining its original

lyophilized and stored. The other half of the original colored Supernatant I was also lyophilized, labelled *alkaline extract* (because of extraction at pH 8.3), and stored. Hence this *alkaline extract* contains Fractions A and B.

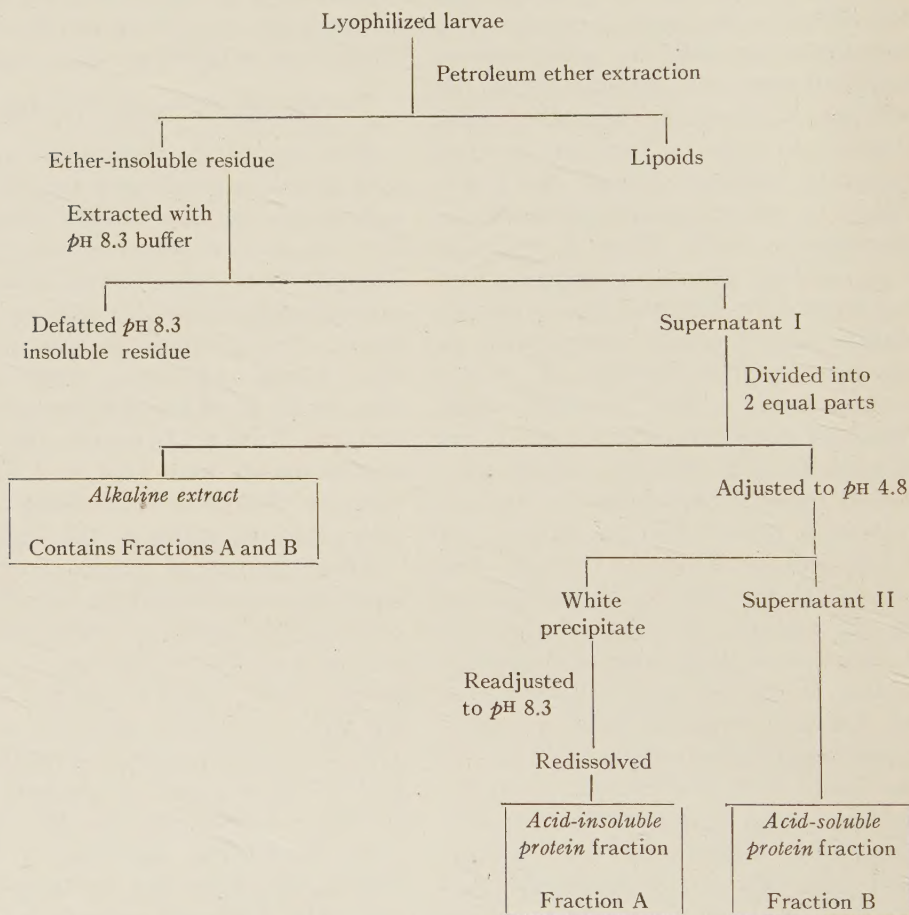


Fig. 1.—Diagram outlining extractions of protein fractions.

color. This precipitate redissolved on treatment with borate buffer pH 8.3. Therefore, this fraction, precipitable at pH 4.8 and soluble at pH 8.3, was designated the *acid-insoluble protein* fraction (Fraction A). It was also lyophilized and stored as above. In contrast, the Supernatant II, soluble at pH 4.8 and 8.3, was labelled *acid-soluble protein* fraction (Fraction B), and was also

The *alkaline extract*, the *acid-insoluble protein* fraction, and the *acid-soluble protein* fraction all gave positive protein tests, namely, biuret, ninhydrin, xanthoproteic, Millon's and sulfosalicylic acid tests.

In summary, 6 fractions were isolated from the original larvae: (1) *polysaccharide*; (2) *lipoids*; (3) *defatted, pH 8.3 insoluble residue*; (4) *alkaline extract*;

- (5) *acid-insoluble protein* fraction; and
 (6) *acid-soluble protein* fraction.

IMMUNOLOGY

Trichinella larvae were obtained from rats by peptic digestion. Eight rabbits (average weight 2 kgs.) were each infected, via stomach tube, with approximately 10,000 of these larvae. Prior to infection, all rabbits used for these experiments were tested for *Trichinella* precipitins and found negative to a titer of 1:10.

Precipitin Tests with Serums from Infected Rabbits

One month after the animals were infected they were bled and their serums again tested for precipitins. Four of the above-mentioned larval fractions were tested for their ability to react with such serums in vitro. They were the *polysaccharide*, the *alkaline extract*, the *acid-soluble protein*, and the *acid-insoluble protein* fractions. Precipitin testing was not practicable with the *lipoid* and the *defatted residue* fractions, because they could not be dissolved in saline. The other fractions tested were easily soluble.

The antigen dilutions were made in relation to the dry weight of the soluble fractions, and not to the weight of the original whole worm material which included insoluble fractions. Earlier workers did not make this distinction. Serial dilutions of the test antigens were made in 0.85% saline. The test mixtures of antigen and antibody (containing 0.5 ml. of undiluted serum and 0.5 ml. of diluted antigen) were shaken, incubated for an hour in a 38 C. water-bath, and then allowed to stand for 12 hours in a refrigerator. At the end of this time the highest final dilution of the antigen which gave a distinct precipitate was considered the titer. The active larval fractions were: the *alkaline extract*,

acid-soluble protein fraction, and the *polysaccharide*. Table 1 shows the results of the reactions between the antiserums and the active larval fractions.

TABLE 1.—*Precipitin Reactions with Serum from Infected Rabbits and Normal Controls*

Serums	Limiting Dilution of Antigens		
	Alkaline Extract	Acid-soluble Protein Fraction	Poly-saccharide
1	1:32,000	1:256,000	1:64,000
2	1:64,000	1:512,000	1:512,000
3	1:16,000	1:128,000	1:32,000
4	1:16,000	1:64,000	1:32,000
5	1:64,000	1:256,000	1:256,000
6	1:32,000	1:128,000	1:32,000
7	1:32,000	1:64,000	1:16,000
8	1:32,000	1:128,000	1:16,000
9*	—	—	—
10*	—	—	—
11*	—	—	—
12*	—	—	—

* Serum from normal control rabbits. These tests were all negative when tested with antigens diluted 1:10.

Results

Of the four soluble fractions tested, the *acid-insoluble protein* fraction produced no precipitates with any of the anti-trichinella rabbit serums. The data listed in table 1 show that the *polysaccharide* and the *acid-soluble protein* fractions are potent precipitating antigens. The *alkaline extract* is merely an earlier stage in the extraction of the *acid-soluble protein* fraction; therefore, it is likely that its precipitating ability is due to the presence of the latter fraction and some *polysaccharide*. The presence of the inactive *acid-insoluble protein* fraction may account for the lower titer of the *alkaline extract* since the total weight of the *alkaline extract* was considered in making the dilutions.

Skin Tests in Infected Rabbits

Skin tests were performed on the same infected animals previously bled for precipitating antibodies. The backs of the animals had been closely clipped before testing. The tests were made 35 days after the animals were infected. All 6 fractions were tested for their skin reactivity, namely, the petroleum

ether-soluble *lipoid* fraction, the *defatted*, *pH 8.3 insoluble residue*, the *polysaccharide*, the *acid-insoluble protein* fraction, the *acid-soluble protein* fraction, and the *alkaline extract*. A suspension of the *defatted residue* and an emulsion of the *lipoids* in saline were necessary before they could be tested; the other fractions were easily dissolved. Each rabbit received six 0.1 ml. injections of each fraction intradermally, a single injection representing 1 dilution of the fraction. The dilutions were made

the fractions of the larvae which account for the skin reaction in trichinella-infected rabbits. A parallelism in immunological response exists between the antigenic fractions active in the skin test and the antigenic fractions active in the precipitin tests, *i.e.*, in both tests the *alkaline extract* reacts in a lower dilution and the *acid-soluble protein* fraction reacts in a higher dilution. This again is probably due to the fact that the *alkaline extract* is an earlier stage in the extraction of the *acid-soluble*

TABLE 2.—Skin Reactions of Infected Rabbits and Normal Controls 24 Hours after Intradermal Administration of Antigens

Antigen		Animals Tested											
		1	2	3	4	5	6	7	8	9*	10*	11*	12*
Alkaline Extract	1:100	+	+	+	+	+	+	+	+	—	—	—	—
	1:500	+	+	+	+	+	+	+	±	—	—	—	—
	1:1,000	±	+	±	+	+	±	—	—	—	—	—	—
	1:5,000	—	—	—	—	—	—	—	—	—	—	—	—
Acid-soluble Protein Fraction	1:100	+	+	+	+	+	+	+	+	—	—	—	—
	1:500	+	+	+	+	+	+	+	+	—	—	—	—
	1:1,000	+	+	+	+	+	+	±	+	—	—	—	—
	1:5,000	+	+	+	+	+	+	+	+	—	—	—	—
	1:10,000	+	+	+	+	+	±	—	—	—	—	—	—
Degree of Infection**		12	14	10	8	17	6	4	9	0	0	0	0

* Normal control rabbits.

** The degree of infection was determined by examining the diaphragms of each rabbit. One hundred low power fields of each were observed and the average number of larvae per field recorded.

+ = Positive skin reaction defined as an indurated erythematous area, the central portion of which became necrotic.

— = Negative skin reaction.

just before inoculation, with sterile technique used throughout. Saline injections were used as controls. Only the fractions which gave positive skin tests are listed in table 2. The animals were sacrificed and autopsied a week later, with the number of encysted larvae in the diaphragm being counted in order to correlate roughly the degree of infection with the intensity of the immunological response.

Results

The skin reactions were of the delayed type, occurring 24 hours after intradermal administration of the antigens. The results, summarized in table 2, show that the *acid-soluble protein* fraction and the *alkaline extract* are

protein fraction and still contains the inactive *acid-insoluble protein* fraction. The presence of the *acid-soluble protein* fraction in the *alkaline extract* accounts for the latter's skin test antigenicity, and the simultaneous presence of the inactive *acid-insoluble protein* fraction accounts for its lower activity per unit weight. The *acid-insoluble protein* fraction obtained from the *alkaline extract* appears to be non-reactive in the intradermal test as well as in the precipitin reaction. Hence, the potent skin test antigen is the *acid-soluble protein* fraction, isolated from the *alkaline extract* by removing the inactive *acid-insoluble protein* fraction.

The *lipoids* and the *defatted residue* gave non-specific reactions in the nor-

mal controls and the infected animals. This may be due to their relative insolubility in saline and in body fluids. The *polysaccharide* in the concentrations indicated was not reactive as a skin test antigen.

*Precipitin Tests with Antiserums
Prepared against Larval
Fractions*

Twelve rabbits were immunized, in groups of 3, with the following 4 antigens: *polysaccharide*, *acid-soluble protein*

made of the 12 possible combinations, the 3 serologically active *Trichinella* antigens, the *Ascaris* antigen, and their antiserums. The tests were carried out as above, using 0.5 ml. of both serum and antigen. The results of these tests are recorded in table 3.

Results

The results of these tests did not indicate the presence of an antigen in the extract of adult *Ascaris suum* in common with the antigens obtained

TABLE 3.—*Precipitin Reactions between Antigens from Trichinella Spiralis and Ascaris Suum and Their Antiserums*

Test Antigen	Antiserums			
	<i>Ascaris</i> Alkaline Extract	<i>Trichinella</i> Alkaline Extract	<i>Trichinella</i> Acid-Soluble Protein	<i>Trichinella</i> Poly- saccharide
<i>Ascaris</i> Alkaline Extract	1:25,600	—	—	—
<i>Trichinella</i> Alkaline Extract	—	1:20,000	1:10,000	1:800
<i>Trichinella</i> Acid-Soluble Protein	—	1:64,000	1:128,000	1:6,400
<i>Trichinella</i> Polysaccharide	—	1:300	—	1:64,000

fraction, *alkaline extract* of *Trichinella* larvae, and an *alkaline extract* of lyophilized, powdered *Ascaris suum* adults. Six injections were given each rabbit, each injection containing 5 mgs. of antigen in 0.5 ml of saline. The injections were given intravenously every other day, except for a rest period of 3 days between the third and fourth injections. Solutions of antigens were made immediately before each injection. Prior to sensitization, these rabbits were bled from the ear vein. Their serums were tested but did not react with any of the fractions in a titer as low as 1:10. The rabbits were bled by cardiac puncture 10 days after the last injection, and the serums from the 3 rabbits receiving the same antigen were pooled. It has been stated that *Ascaris* contains an antigen in common with *Trichinella*,²⁷ and serological tests were made to determine this. Precipitin tests were then

from the *Trichinella* larvae. The reactive fractions are complete antigens. The *polysaccharide* was precipitinogenic, reacting in a dilution of 1:64,000 with its homologous antiserum. The *acid-soluble protein* fraction precipitated its homologous antibody to a titer of 1:128,000; the *Ascaris* and *Trichinella alkaline extracts* gave positive precipitin tests in dilutions of 1:25,600 and 1:20,000 respectively, with serums prepared against them. Points to consider are the relatively high concentration of *polysaccharide* (1:300) required to precipitate antiserum against the whole worm extract, and the high concentration of the whole worm extract (1:800) necessary to precipitate *polysaccharide* antiserum. This suggests that the *polysaccharide*, when in combination with protein (as it probably is, since all the protein fractions above gave positive Molisch tests), is masked by the protein and plays an insignificant role in the stimulation of *polysaccharide*

27. Baron, B., and Brunner, M.: J. Allergy. **13**: 459, 1942.

antibody. This may account for the negative reaction between the *polysaccharide* and the serum prepared against the *acid-soluble protein* fraction. The *polysaccharide* exists as a complete antigen in the protein-free state, inducing the formation of, and precipitating with homologous antibodies. Assuming that

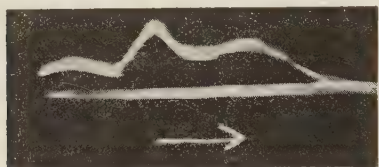


Fig. 2.—Electrophoretic diagram of the acid-soluble protein antigen.

the *polysaccharide* in combination with the protein acts like a complex haptene (precipitating serum prepared against the isolated *polysaccharide*), then the cross reaction between the antipolysaccharide serum and the *acid-soluble protein* fraction can be explained.

ELECTROPHORETIC PATTERN OF THE ACID SOLUBLE PROTEIN FRACTION

The data obtained from the precipitin reactions and skin tests in the experimentally infected rabbits indicate that the *Trichinella* larval antigen responsible for the positive reactions in both of these tests is the fraction here designated as the *acid-soluble protein* fraction. The electrophoretic pattern and migration rates of proteins are considered established physical constants; therefore, this method of analysis was applied to the immunologically active protein fraction isolated here. This antigen was studied in the Tiselius apparatus by the moving boundary method.²⁸ A 2% solution of the antigen was equilibrated against phosphate buffer, pH

7.7, ionic strength 0.1, and then placed in the apparatus. The potential gradient was 4.2 volts per cm., the temperature was maintained at 1 C. and the optical system used was that of Philpot and Svensson.^{29,30,31} In figure 2 is shown the electrophoretic diagram of the descending boundary of this protein fraction. The curve was photographed after migration for 120 minutes. The fraction consists of 3 distinct migratory peaks, the mobilities of which were -0.32×10^{-5} , -1.8×10^{-5} , and -3.7×10^{-5} cm.²/volt/sec. The percentage compositions for the 3 components were respectively 24%, 35%, and 41%.

The extremely low mobility of the slowest component suggests that it may be a polysaccharide. Furthermore, this trimobile fraction gives a positive Molisch test.

DISCUSSION

In vivo and in vitro immunological tests indicate that the fraction called the *acid-soluble protein* fraction is a potent antigen of the larvae of *Trichinella spiralis*. It was isolated by alkaline extraction of dehydrated, defatted larvae and purified by acid precipitation of the non-reactive substances. Because this antigen is soluble in both acid and alkaline pH ranges, and appears to be the only fraction possessing both skin test and precipitating activity, it is probably the fraction responsible for the positive skin tests and serological reactions described by previous investigators working with less refined extracts of the larvae.^{5,6,7,19,20} This is interesting in reference to the recent work of Oliver-González²² who

29. Philpot, J. S. L.: Nature, London **141**: 283, 1938.

30. Svensson, H.: Kolloid-Beihefte **87**: 181, 1939.

31. Svensson, H.: Kolloid-Beihefte **90**: 141, 1940.

28. Tiselius, A.: Trans. Faraday Soc. **33**: 524, 1937.

showed that the antilarval antibody was absorbed from immune rabbit serum by ground whole *Trichinella* larvae. No doubt the absorbing power of the latter is largely due to the *acid-soluble protein* fraction described above. However, this does not preclude the existence of other antigenic fractions. The electrophoretic pattern of this antigenic fraction is relatively simple—only 3 components are present at the pH and ionic concentration of the buffer employed. Data on the electrochemical character of the *acid-soluble protein* fraction is offered as a means of standardizing *Trichinella* antigenic preparations, because in this analysis of the larvae it is the fraction which is responsible for both high precipitating and skin test activity.

The polysaccharide, reacting as a precipitinogen, also plays a role in the antigenic mosaic. It did not, however, give skin tests.

The lipoidal fraction remains to be studied by complement-fixation or active immunization to determine its *in vitro* or *in vivo* antigenic potentialities; however, when used as a skin test antigen, it produces only non-specific reactions.

SUMMARY

The relative antigenicity of the different fractions of the larvae of *Trichinella spiralis* was studied. An *acid-soluble protein* fraction consisting of 3 electrophoretic components was isolated. Positive skin tests were observed in infected rabbits with 20 $\mu g.$ and positive precipitin reactions with 2 $\mu g.$ of this fraction. This protein fraction was a complete antigen inducing antibodies in rabbits, and reacting with these antibodies. These antibodies did not cross-react with antigenic material from *Ascaris suum* adults.

A *polysaccharide* isolated from the *Trichinella* larvae gave positive precipitin reactions in high dilutions (2 $\mu g.$) with serums of infected rabbits, but did not give a skin test under the conditions of these experiments. However, this *polysaccharide* induced precipitins in rabbits, and the serological data presented suggest that it may be associated with the potent *acid-soluble protein* antigen.

The author wishes to express his appreciation to Professor D. H. Campbell for his suggestions and advice during the course of this work, and to Dr. G. G. Wright for assistance in the electrophoretic analysis.



